A COMPARATIVE STUDY OF THE CUTICULAR STRUCTURE OF THREE FEMALE MEALY BUGS (HOMOPTERA: PSEUDOCOCCIDAE)

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The recent discovery in the arid north-west of South Australia of a new species of the hitherto monotypic genus, *Epicoccus,* afforded an opportunity to examine the cuticle of a drought-resistant pseudococcid. Members of the Pseudococcidae are normally confined to microhabitats where they are surrounded by humid equable conditions, and it was anticipated that considerable modification of cuticular structure would be shown by a species which has evolved in an area where it is fully exposed to the desiccating effects of high temperatures, low relative humidities, and drying winds. The extent of such modification, if any, could be gaged only after the cuticle had been compared with that of a typical form, of which, however, there appears to be no published account. A study of the cosmopolitan long-tailed mealy bug, *Pseudococcus adonidum* L., was therefore undertaken as a preliminary step in the investigation. Finally, the cuticle of the one described species of Epicoccus, *E. acaciae* (Maskell) was examined to ascertain whether any major differences in cuticular structure occur within the genus.

The terminology is in accord with the scheme which I recently outlined (Lower, 1956).

Materials and Methods

Specimens of *P. adonidum* were obtained locally from a heavily-infested plant of *Daphne odora.* Those of *Epicoccus* sp., were collected from *Acacia aneura* F. Muell., at Yudnapinna in the north-west of South Australia while those of *E. acaciae* came from the coastal strip of Western Australia, the only area in which the species is known to exist.

The insects were first killed with cyanide, and free-hand sections of some of each species at once stained with Sudan black B. The remainder, after fixation in Sanfelice's fluid, were embedded, part in a water-soluble wax and part in paraffin. Sections cut from these were similarly stained. Comparison showed that there was no observable loss of cuticular lipoid when paraffin was used as the embedding medium. All work was therefore done using paraffin sections cut at 4 μ and 1 μ, except that when the external wax of *Pseudococcus* was being investigated, sections prepared by the first two methods were used.

The histochemical tests and techniques applied have been described elsewhere (Lower, 1957a).

I. The Cuticle of Pseudococcus adonidum L.

The cuticle (Fig. 1) is thin, measuring in most parts between 6 μ and 8 μ; only exceptionally does it attain a thickness of 10 μ. Its structure is relatively un-

specialized and except for the brown outer layer of the epicuticle it is unpigmented. Pore canals, if present, could not be observed with the light microscope either under bright-field or phase-contrast conditions. A thick layer of wax (discussed later) covers the cuticular surface.

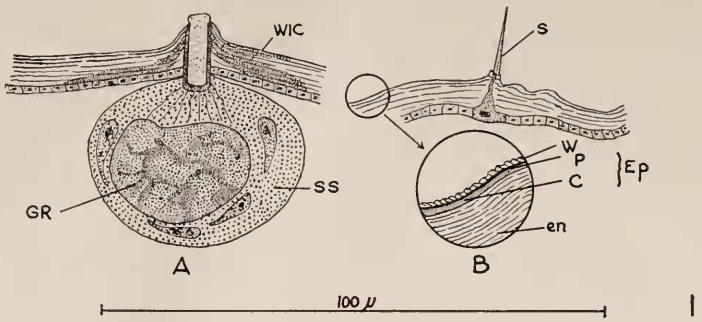

FIGURE 1. *P. adonidum:* Cuticle. A. Part of cuticle adjoining glandular duct. B. General cuticle. c, cuticulin layer; en, endocuticle; Ep, epicuticle; GR, gland reservoir; P, paraffin layer; S, spinule; SS, secretory sheath; W, surface wax layer; WIC, wax-impregnated cuticle.

A. The Epicuticle

The epicuticle (Fig. 1B) is two-layered and has a total thickness of about 1 μ, of which the inner layer constitutes the greater part.

The inner or cuticulin layer is colorless and transparent. It responds positively to the Millon, xanthoproteic and biuret tests, is non-argentaffin, and is unaffected by either Sudan black B or Nile blue sulfate. It is stained red by the routine stain and pink by Sevki's diluted Giemsa technique, but it cannot be stained either by Mallory's PTAH or any of the iron haematoxylins. Schmorl's test shows the absence of reducing substances. It is soluble in warm concentrated solutions of potassium hydroxide or hydrochloric acid. Its location and general chemical reactions indicate that it is probably homologous with the cuticulin layer of *Rhodnius* (Wigglesworth, 1947).

The thinner outer layer is brown-pigmented. The color is difficult to discharge, sections requiring about a week's immersion in 10% hydrogen peroxide to effect this. The strong positive response to Schmorl's test, together with the non-argentaffin nature of the layer, suggests that the coloring matter is a lipofuscin. The layer responds to none of the protein tests. It is blackened by Sudan black B, and stained deep blue by Cain's Nile blue sulfate technique. Prolonged differentiation during the latter process removes much of the blue without any red appearing so that acidic lipoid only appears to be present. It is stained black by iron haematoxylins and deep violet by Mallory's PTAH. It is resistant to concentrated solutions of potassium hydroxide and hydrochloric acid but fuming nitric acid or *aqua regia*, when gently warmed, attacks some of its constituents and liberates a material which is practically instantaneously soluble in cyclohexane and is quickly and easily stained by any of the fat stains. Its anatomical position and general chemical behavior indicate that the layer is a paraffin epicuticle (Dennell and Malek, 1955). It differs from the corresponding layer in Sarcophaga (Dennell,

1946) by being non-argentaffin, in giving no response to the xanthoproteic reaction, and in its content of lipofuscin pigment.

B. The Procuticle

Practically the whole of the procuticle is present as endocuticle. With the exception of the spinules mentioned below, no exocuticle occurs, while the mesocuticle is confined to the alveoli of the spinules and the small convex circular patches (appearing crescentic in transverse section) where muscles are inserted in the cuticle.

Scattered over the cuticular surface are weakly-sclerotized, colorless spinules (Fig. 1B), each secreted by a trichogenic cell which is much larger and more granular than the adjacent hypodermal cells. A cytoplasmic process from each trichogenic cell, after passing through the cuticle, attenuates and forms a core to the spinule for its basal two-thirds. Both spinules and alveoli are feebly argentaffin and are stained by the routine stain, the former a very pale pink and the latter red.

The endocuticle is laminated and closely resembles that found in other insects with soft cuticles. Its principal constituent is the normal chitin-protein complex.

II. The Cuticle of Epicoccus sp.

To comprehend the structure of the cuticle of *Epicoccus*, some knowledge of its mode of development is essential.

At the end of a short period of wandering, the second female nymphal instar assumes a permanent position on a twig, petiole, or leaf, moults, and enters her third and final stadium. Rapid development of the cuticle now begins. The cells of the dorsal hypodermis hypertrophy and their secretory activity correspondingly increases. This gives rise to enormous allometric growth of the dorsal cuticle as two lobes, one along either side, whose inner surfaces are in close contact with the bark of the host. Posteriorly these do not clasp the stem but grow round until their margins meet enclosing a small orifice (Fig. 3). While these changes have been in progress, glands located along the parts of the lobes contiguous with the bark have been pouring out large quantities of wax, not unlike bees-wax in colour and texture, which cements the insect firmly to the surface of the stem. Meanwhile, its ventral surface has moved outwards away from the bark, the space so formed constituting a brood chamber (Figs. 2 and 3) whose only means of communication with the exterior is through the pore between the adpressed posterior tips of the lobes. As a result of these changes, the greater part of the cuticle, and the only part visible when the insect is *in situ*, consists of the large thickened dorsum. The remaining much-smaller enclosed region comprises the small thin sternal cuticle and the relatively-minute, degenerate pleural regions. The whole development resembles much more closely that of a coccid than that of a pseudococcid. The thickened dorsum, deeply infolded to expose the least possible area to the atmosphere, the enclosure of the entire thin part of the cuticle, and the spiracles opening into the brood chamber, all appear to be adaptations to minimize water loss.

The fully-developed female (Fig. 4) has an average length of about 3 mm. and is somewhat less than this in width. Transverse sections display a great variety of contour, the only consistent feature being their distorted U-shaped outline. Although an occasional isolated female may be almost bilaterally symmetrical, the normal condition, brought about by the gregarious mode of life, is for one or the

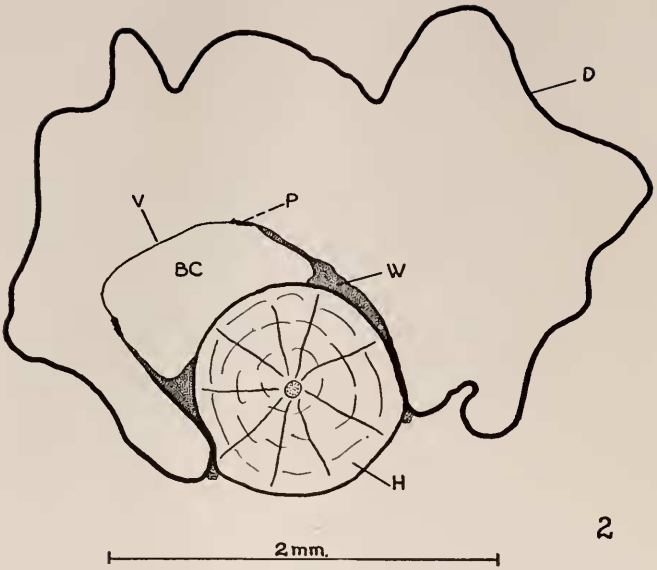

FIGURE 2. *Epicoccus* sp. Transverse section showing spatial relation between insect and host. BC, brood chamber; D, dorsal cuticle; H, host; P, pleural cuticle; V, ventral cuticle; W, cementing wax.

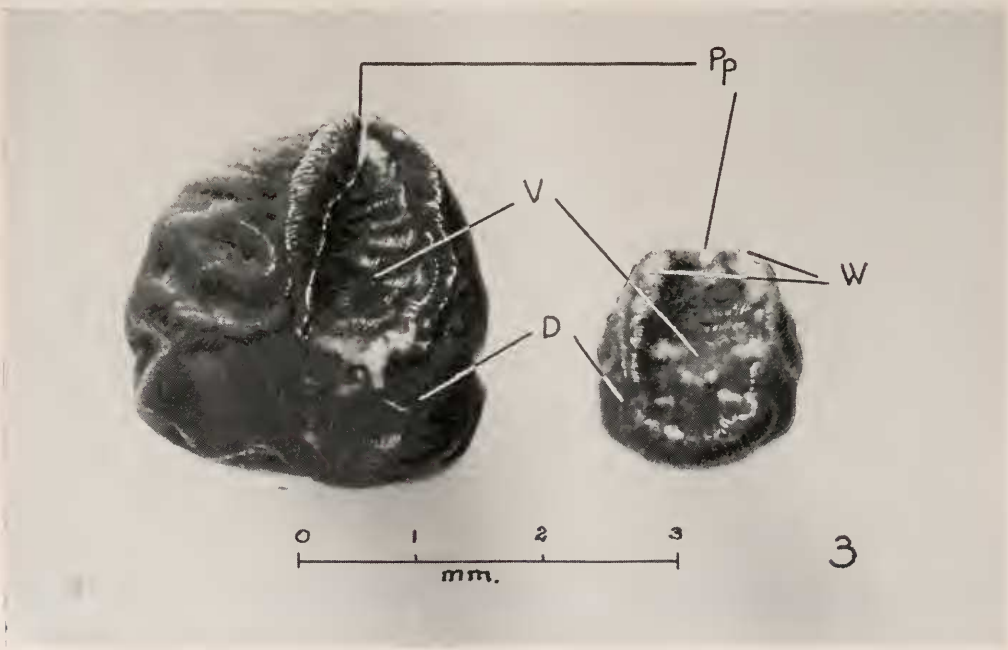

FIGURE 3. *Epicoccus* sp. Fully developed and immature third female instars. Ventral aspect showing brood chamber and posterior pore. D, dorsal cuticle; Pp, posterior pore; V, ventral cuticle; W, cementing wax.

FIGURE 4. *Epicoccus* sp., *in situ*. *Photograph courtesy of Helen M. Brookes.* The females are invariably so oriented that the rounded cephalic end is directed towards the host's region of growth. Part of the cementing wax is visible on the second insect from the top.

other lateral lobe to be more developed than its fellow. Which of these is the larger is determined by the proximity of neighboring females, leaf petioles or similar obstructions, and the natural asperities of the bark encountered during growth. Figure 2 was drawn from a section selected, not because it was typical, but because it clearly displayed the spatial relation between insect and host.

The mouthparts excepted, the only regions of the body capable even of limited movement are the pleura and the ventral abdominal surface, both of which are subordinate to the functions of oviposition and defaecation.

The cuticle exhibits great diversity of thickness, structure and composition. For descriptive purposes, four major types are here recognized: the respective cuticles of the dorsum, the venter, the pleura, and the intersegmental membranes. The latter are restricted to the enclosed ventral surface; externally, they are suppressed by fusion of the segments and are represented only by sutures.

1. *The Dorsal Cuticle*

Relative to the size of the insect, the dorsal cuticle is massive, ranging in thickness from a minimum of 30 μ near its junction with the pleura (Figs. 5 and 6A) to some 40 μ in the remainder (Fig. 6C). Maximum thickness is attained in small localized patches where muscles, particularly those in the abdomen associated with the organs of oviposition, are inserted. In such areas thickenings of 70 μ or more are not uncommon (Fig. 6B). It exhibits a general uniformity of structure throughout, consisting of a two-layered epicuticle overlying the procuticle. It is interesting to note that no part of the cuticle is argentaffin.

A. The Epicuticle

Irrespective of its location, the epicuticle displays a constancy of thickness, structure, and composition so that what is said of it here in connexion with the

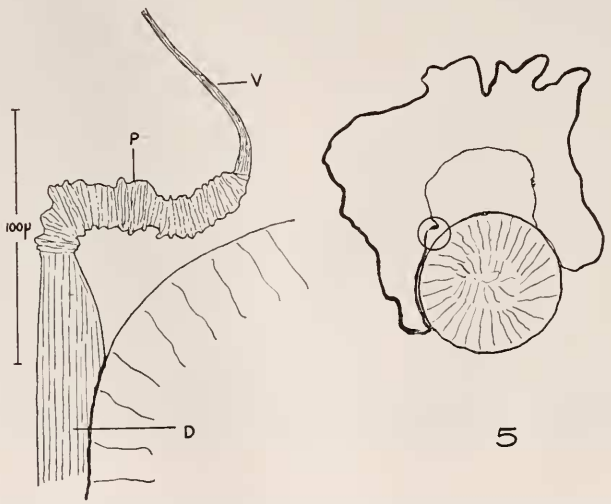

FIGURE 5. *Epicoccus* sp. Dorso-pleural and ventro-pleural junctions. The part of the cuticle drawn is indicated by the small circle in the diagram at right. Symbols as for Figure 2.

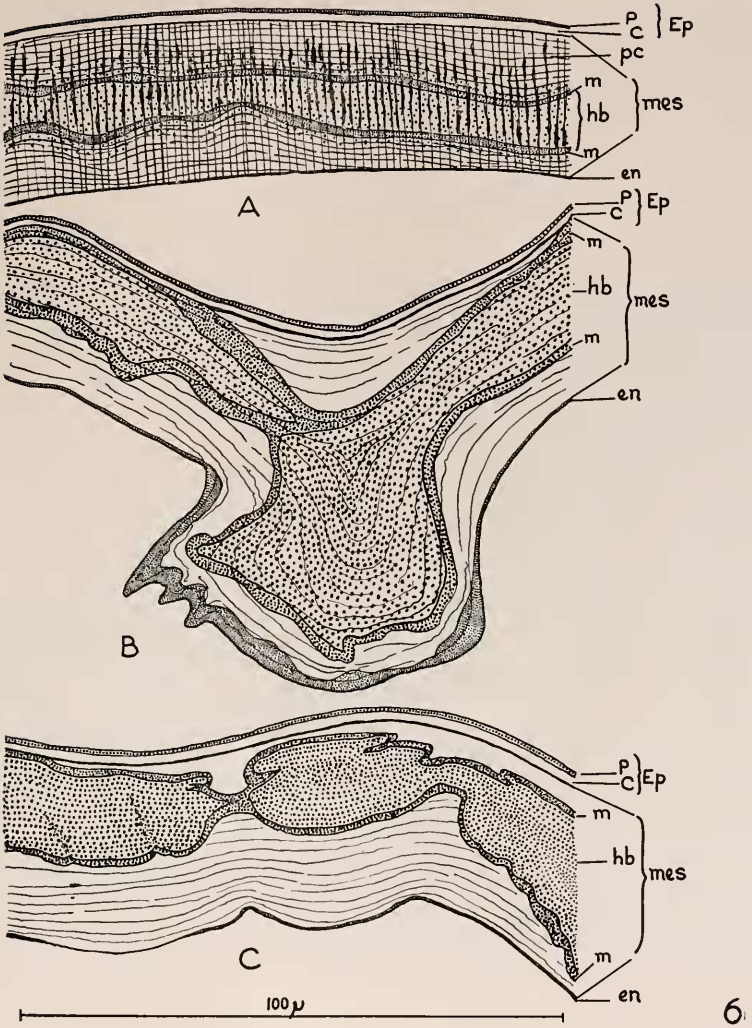

FIGURE 6. *Epicoccus* sp. Dorsal cuticle. A and C, general cuticle; B, cuticular ingrowth. Pore canals are omitted from B and C for clarity. hb, heavily-staining zone; m, margins of heavily-staining zone; mes, mesocuticle. Other symbols as for Figure 1.

dorsal cuticle has general application. Except for the non-pigmented condition of the paraffin layer, it is practically indistinguishable from the same structure in *Pseudococcus*. It has a total thickness of about 1.5 μ distributed between the (outer) paraffin component which constitutes about one third of it, and the (inner) cuticulin layer which comprises the remainder (Figs. 6 and 7).

The Cuticulin Layer:

The cuticulin layer is colorless and transparent. It gives a vigorous response to Millon's reagent, is stained yellow by the xanthoproteic reaction, and orange-

red by the routine stain. It is non-argentaffin, non-reducing, non-iodophil, and is unaffected by Danielli's, Gibb's, or Mallory's PTAH techniques. It gives negative responses to Sudan black B and Nile blue sulfate. It is easily soluble in hot solutions of potassium hydroxide but resists for at least twelve hours the action of 10% hydrochloric acid at 60° C. These responses indicate a composition largely proteinaceous. The materials to which the protein is bound are such that the usual range of tests do not serve for their identification. They also confer on it its resistance to extraction by hot dilute acids.

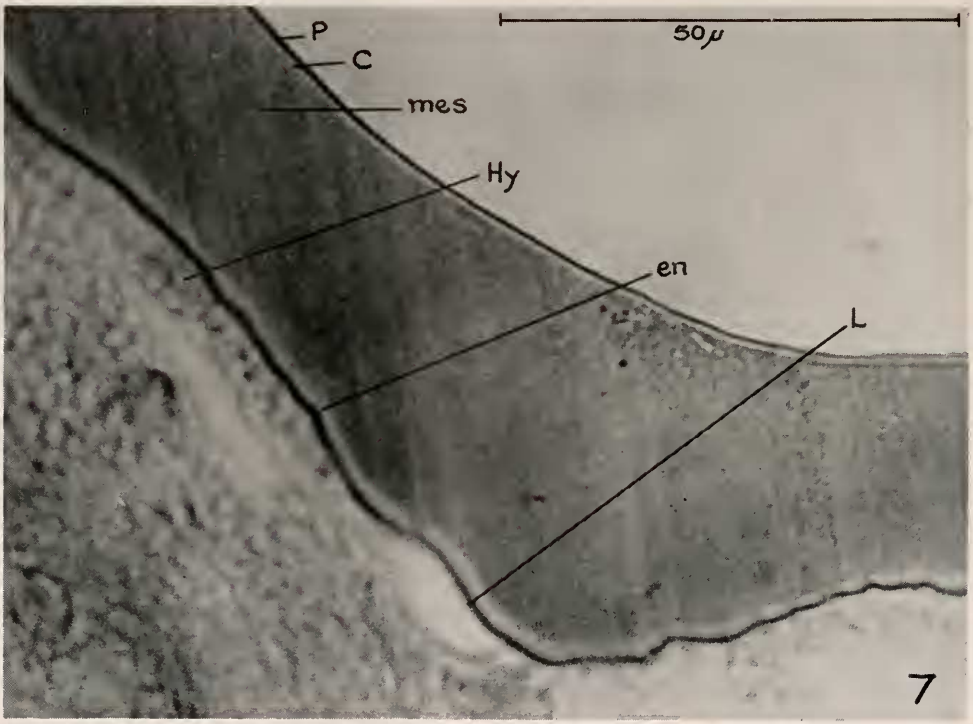

FIGURE 7. *Epicoccus* sp. Dorsal cuticle 4 μ; stained with Sudan black B, bright-field. Hy, hypodermis; L, lipoid zone; mes, mesocuticle. Other symbols as for Figure 1.

The Paraffin Layer:

This layer is colorless. Its characteristic component is lipoid, as the strong positive responses to fat stains (Fig. 7) and osmic acid indicate. Schmorl's test shows it to possess strong reducing properties and Mallory's PTAH stains it deep violet. It is intensely iodophil but it is not affected by the other reagents used nor is it soluble in dilute acids or alkalis.

The Surface Secretion:

The dorsal cuticle of living insects or of those killed with cyanide has a resinous luster (Fig. 4) which is retained when the dead specimens are dehydrated. The

glossy surface cannot be wetted. If freshly killed or dried specimens are treated with fat solvents, 90% ethanol, or warm 5% sodium or potassium hydroxide solution for five minutes, and then washed with water and dried, the luster is lost and the entire surface becomes dull. Treatment with boiling water, hot concentrated hydrochloric acid, or exposure to dry heat at 100° C. for twelve hours does not affect it. Immersion of insects in molten water-soluble wax similarly leaves the luster undimmed.

Untreated and "dulled" insects were therefore embedded in this medium, the sections stained with aqueous dyes in acid solution, and mounted in glycerol which is without effect on the luster. Sections cut from both batches were indistinguishable nor did comparison with sections of fresh material reveal any recognizable differences.

This scanty evidence suggests that a layer of sub-microscopic thickness (possibly containing lipoid) may cover the paraffin layer of the dorsal epicuticle.

B. The Procuticle

The procuticle consists almost entirely of mesocuticle (Fig. 7). Exocuticle is restricted to small widely-dispersed papillae, each bearing a minute hemi-sclerotized seta. The endocuticle forms a narrow zone about 1 μ in thickness except at the termination of cuticular ingrowths where its lobes and thickened portions serve as intermediaries for muscle attachment (Figs. 6 and 7).

The Exocuticle:

In the sense that exocuticle is completely-sclerotized procuticle, this zone is wanting in *Epicoccus*. Both papillae and setules are transparent; the former are very pale yellow, the latter are colourless. Neither is inert to aniline stains as is true exocuticle. The routine stain colours both of them pink and the colour deepens as time of immersion is increased. Treatment for a few minutes with "Diaphanol" is sufficient to destroy the incipient sclerotization and they then stain intensely and rapidly with acid dyes. Their development appears to be in a stage intermediate between mesocuticle and exocuticle. Complete sclerotization commonly induces changes in the overlying cuticulin layer but the epicuticle of the papillae differs in no respect from that of other parts of the body.

The Mesocuticle:

The mesocuticle shows little structural differentiation. When unstained sections are examined under the highest powers of the light microscope the mesocuticle appears uniform and featureless. Under phase-contrast conditions it is transversely marked with numerous, irregular dark streaks which indicate the positions of pore canals of whose organization, however, no details are observable. The routine stain dyes the zone red but supplies little information on canal structure,

Chemically, the mesocuticle is as complex as it is structurally simple. Millon's reagent differentiates it into three clearly-defined sub-zones which, beginning with the outermost, are here referred to as the A, B, and C sub-zones, respectively (Fig. 8).

The A sub-zone is stained pink by Millon's reagent, and the distal parts of the pore canals appear as indistinct, thin red lines which, after traversing the region,

attenuate before they terminate at its outer surface. It is of variable width; in some parts it may form as much as a third of the cuticle but is generally less. Occasionally it is suppressed by the outward extension of the B sub-zone (Fig. 6B).

The B sub-zone takes the form of a conspicuous, broad, bright-red band, sharply demarcated externally and internally from the remainder of the procuticle by narrow crimson margins (Figs. 6 and 8). It is continuous throughout the dorsal mesocuticle, though it thins before it terminates at the dorso-pleural junctions. This intensely-staining proteinaceous region exhibits great and sudden variations in thickness. Its maximum development may be observed in cuticular ingrowths of which it occupies the greater part (Fig. 6B). Where it is thinnest (Fig. 6C), it is composed solely of the contiguous crimson margins. The distinct differences

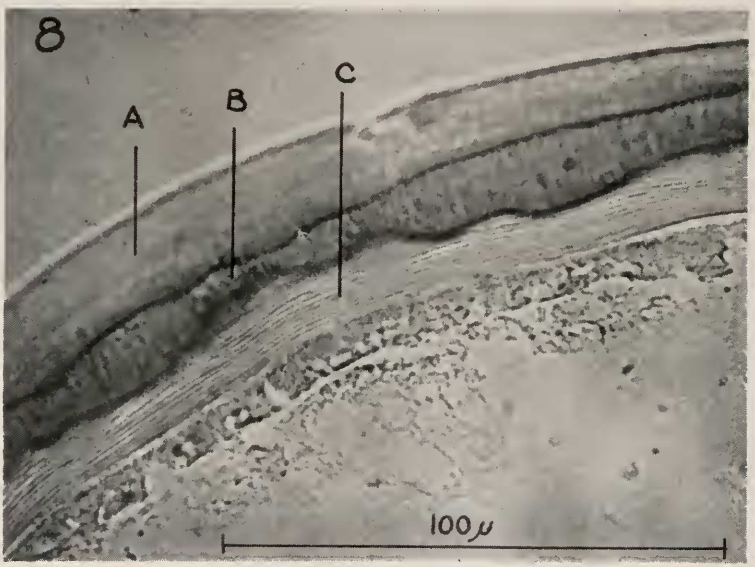

FIGURE 8. *Epicoccus* sp. Dorsal cuticle, 4 μ. Millon's reagent; bright field. A, B, and C are the three sub-zones referred to in text. Maximum definition in the mesocuticle has been sought; the epicuticle is distorted through being out of focus.

in color between the central part and its marginal bands suggest either that different proteins are present in each or that the concentration of the one protein is higher in the margins than internally. The general appearance is reminiscent of the result obtained by the use of the paper-chromatography technique. The great mass of the pore canals (Fig. 9) is located within the zone and it is possible that a protein complex in solution diffuses from them into the circumjacent cuticle there to undergo partial separation, the chitinous matrix acting similarly to the chromatographic paper.

Sub-zone C is stratified in alternate red and pale-pink layers (Fig. 8). Whether or not these coincide with the probable original lamellate deposition of the procuticle cannot be determined since no other stain or technique used, produced comparable differentiation. This sub-zone varies in thickness but occupies about the inner third of the mesocuticle.

Of the wide range of tests applied to the mesocuticle none so clearly distinguished the sub-zones as did Millon's reagent. Other tests which responded positively gave diffuse results, but confirmed the fact that the concentration of protein in the B sub-zone is higher than in any other part of the mesocuticle. Ninhydrin colored the mesocuticle violet-pink, the greatest depth of color being developed in the B sub-zone. The xanthoproteic reaction stained it deep orange medially, paling to yellow towards either surface. The iodine technique (Lower, 1957b) approximately delineated the sub-zone by staining it deep purple-black, the C sub-zone was uniformly dark red, and the A sub-zone was practically unstained. Mallory's PTAH produced a similar picture, the B sub-zone being reddish violet with deeper violet margins, the C sub-zone light red, and the A sub-zone almost colorless.

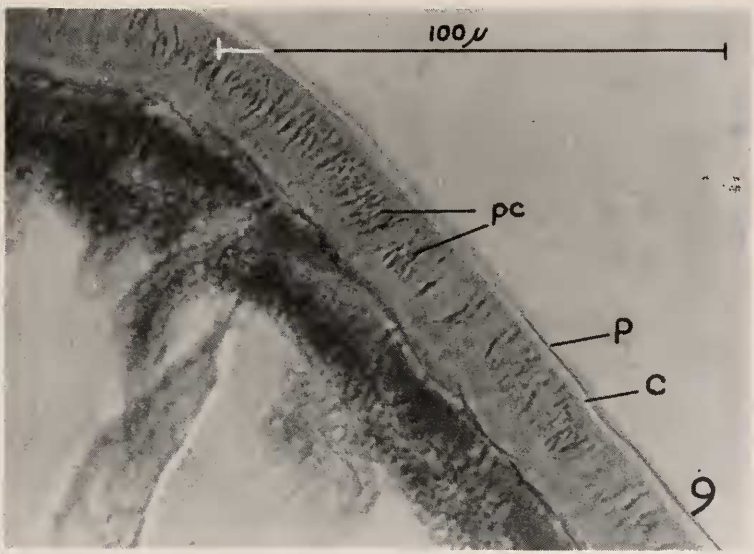

FIGURE 9. *Epicoccus* sp. Pore canals in latero-dorsal cuticle. One micron; stained by Sevki's technique; bright-field. Note termination of pore canals at outer mesocuticular surface. C, cuticulin layer; P, paraffin layer; pc, pore canals.

As is usually the case, Sevki's technique produced the clearest differentiation of the pore canals (Figs. 9, 10). Their thin hypodermal connections were colored red, their thickened portions in the B sub-zone were deep purple, and their thin terminal parts in the A sub-zone were red. Their numerosity is such that even in sections cut at 1 μ they appear as a confused mass (Fig. 10). The only valid conclusions that can be drawn concerning them are the following: the pore canals are confined to the procuticle, being continuous between its outer surface and the hypodermis; they are extremely numerous, of highly irregular form, and the mass of their contents, which possess a high concentration of tyrosine-containing protein, is almost entirely located within the B sub-zone.

The mesocuticular protein is separable into two fractions. Sections were immersed for twelve hours in 10% hydrochloric acid maintained at 60° C. After

washing in distilled water it was found that the mesocuticle had lost its characteristic staining properties. Millon's reagent colored it uniformly pink and the xanthroproteic reaction pale yellow. Ninhydrin, Mallory's PTAH, and the Sevki and iodine techniques all gave negative results. The routine stain dyed it light red. In no section were pore canals visible and even the use of phase-contrast failed to reveal their positions. These results demonstrate that while most of the protein is extractable with hot dilute hydrochloric acid, there is a residual fraction firmly bound to the chitin in such a manner that its extraction is more difficult.

There is no evidence to suggest either the mode of accumulation or functioning of the apparently high protein concentration in the B sub-zone. The adult male of either species of *Epicoccus* is unknown; the cuticles of first and second nymphal

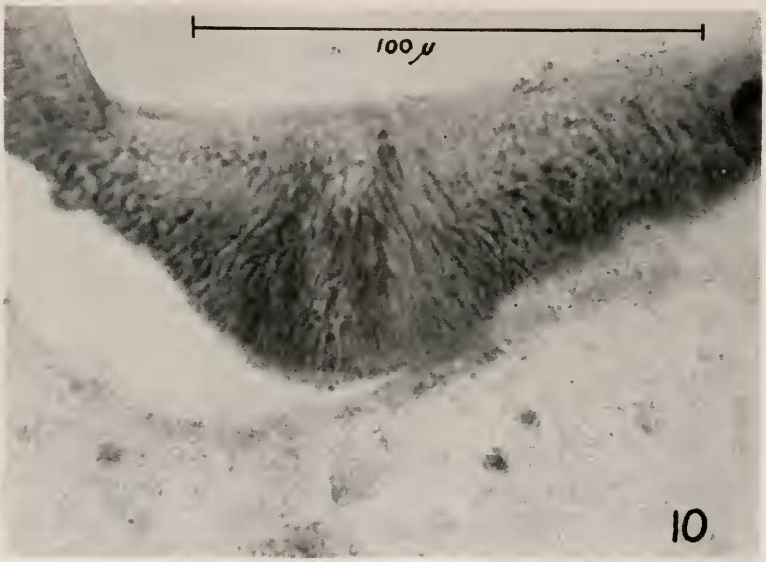

FIGURE 10. *Epicoccus* sp. Pore canals in thick part of dorsal mesocuticle. One micron; stained by Sevki's technique; bright field.

instars of either sex do not differ significantly from those of the corresponding instars of other pseudococcids. Of the known stages, therefore, the phenomenon is confined to the third female instar in which the thickness of the sub-zone relative to that of the cuticle as a whole increases with the age of the insect.

The Endocuticle:

As mentioned above, the endocuticle is greatly reduced. It is wanting in many parts of the cuticle, and, where present, is never more than 1 μ in thickness except where it caps the cuticular ingrowths. Its structure appears normal.

The Lipoid Zone:

When the routine stain is applied to a cuticular section, a thin layer, staining a much deeper green than does the endocuticle, can be seen to separate the latter

from the hypodermal cells. This layer has an average thickness dorsally of about 1 μ, and is continuous throughout the dorsal cuticle except where interrupted by cuticular ingrowths. Laterally, it attenuates until it disappears in those parts of the dorsal lobes which are in contact with the bark. It is absent from the cuticles of the pleura, venter, and intersegmental membranes. In unstained sections, the layer is indistinguishable from the endocuticle, since the structure and natural color of both are the same. Its high content of chitin shows it to be definitely of procuticular origin, and but for its impregnation with other materials, it would merely represent the innermost part of the endocuticle.

It is remarkable for its high lipoid content, and to obviate additions to the terminology, unjustifiable at this stage, it is referred to descriptively as the *lipoid zone*. The lipoid zone has unusual chemical properties for a part of the procuticle, so anatomically located. In addition to the reactions characteristic of endocuticle generally, it is stained deep violet by Sevki's technique and Mallory's PTAH. It is most intensely colored, however, by the fat stains.

In free-hand sections of fresh material, the zone can be deeply stained at room temperature by short immersion in the fat stains. Sections cut from paraffin-embedded material, or those cut from cuticles which have been repeatedly extracted with boiling cyclohexane, cannot be stained in this manner. Nearly as intense color, however, can be developed by immersion for several hours at 60° C. in the same stains. The Nile blue sulfate technique shows that part, at least, of the lipoid is neutral, since the zone is stained red after differentiation. If sections cut from extracted or paraffin-embedded material be gently warmed with fuming nitric acid, the chitin-lipoid association is destroyed, and the previously-dispersed lipoid aggregates into minute droplets which stain rapidly and intensely with fat stains. These results indicate that a lipoid complex, rather than a single lipoid, is involved. Part of the lipoid is free, and easily extractable; part is bound to the chitin and the other constituents of the procuticle of the region, and resists extraction.

Of the origin and function of the lipoid zone, nothing is known. Examination of females in various developmental stages shows that lipoid impregnation synchronizes with secretion of the procuticle, and that the zone maintains its position relative to the hypodermis throughout the development. It is not present in the first and second nymphal instars, and was absent from one very young third female instar which had reached this stage only about the time that the insects were collected. In all the other females examined, no measurable difference in its thickness was observed, and in two exceptionally large specimens (both parasitized), the zone was of normal thickness.

Only twice previously has the presence of a layer between the endocuticle and the hypodermis been recorded in the literature. The two records are those of Schmidt (1956) and Malek (1956).

Schmidt reported that, in certain insects which he had examined, a glycoprotein layer, the "sub-cuticle," separated the endocuticle from the hypodermis. As he stated categorically that the "sub-cuticle" was non-chitinous, it clearly has no affinity with the lipoid zone of *Epicoccus*, and need not be further discussed here.

Malek demonstrated that when the desert locust, *Schistocerca gregaria*, is moulting, the inner part of what was originally endocuticle becomes impregnated with a lipo-protein complex, to form the ecdysial membrane of the insect.

This membrane and the lipoid zone of *Epicoccus* appear to be homologous struc-

tures. Since both are derived from the innermost part of the procuticle contiguous with the hypodermis, both occupy corresponding anatomical positions. Both are impregnated with lipoid, and both, by reason of their derivation, contain chitin.

The stages of the life cycle in which each is present are, however, completely reversed. In *Schistocerca*, the lipoid-impregnated procuticle must, by forming an ecdysial membrane, be necessarily confined to immature stages, since the adult does not moult. The lipoid zone of *Epicoccus*, on the contrary, is found in the adult only. Few details are given in Malek's preliminary note, and it will be necessary to await his full account, before an adequate comparison of the two structures can be made.

2. *The Ventral Cuticle*

The cuticle of the venter is thin, averaging some 4 μ in thickness. Its epicuticle is indistinguishable from that of other parts of the body.

A. The Procuticle

There is no exocuticle. Less than half of the procuticle consists of mesocuticle which displays no signs of the chemical sub-zonation characteristic of the dorsal mesocuticle. It has no visible internal structure and chemically, is typical of this zone generally.

The endocuticle comprises rather more than half of the procuticle, being thicker than that of the dorsum. No lipoid zone is present. Both chemically and structurally it resembles the endocuticle of *P. adonidum*.

3. *The Pleural Cuticle*

What is here assumed to be the cuticle of the degenerate pleura covers two narrow regions, one on either side, which connect the ventral and dorsal cuticles. From both of these the pleural cuticle differs greatly in structure. In transverse sections, its surfaces are irregular (Fig. 5) and its general appearance suggests that it is in a contracted state; it is possible that in the living insect it may be much more extended and correspondingly thinner.

It is frequently thick, exceeding 30 μ in a few places. The epicuticle is typical. The procuticle consists wholly of endocuticle which, when stained, shows no structure under bright-field conditions. When viewed under phase-contrast conditions, whether stained or not, numerous, fine, approximately-transverse dark lines can be seen. These appear to be artifacts produced by the contraction assumed to have occurred. The change from pleural to dorsal, or pleural to ventral, cuticles is sharp; there is no gradation of one region into the other.

Tests reveal the presence of the normal chitin-protein complex; there are no unusual components.

4. *The Cuticle of the Intersegmental Membranes*

Functional intersegmental membranes are found uniting the ventral abdominal segments only, so that cuticle of this kind is restricted to the ventral surface.

It is very thin and consists of the typical epicuticle overlying a procuticle rarely

exceeding 2 μ in thickness and often being less. Its highly plicate condition in many places shows that it permits of considerable movement of the ventral region. The procuticle, which is composed wholly of endocuticle, has no visible internal structure, and no peculiar chemical properties. There appear to be no pore canals in it.

III. The Cuticle of E. acaciae (Maskell)

The cuticle of *E. acaciae* differs in details, only, from that of its congener. The species is somewhat smaller than is *Epicoccus* sp. but relative to the size of the insect its cuticle is still massive.

The epicuticles of both forms are indistinguishable even to the extent that dorsally the paraffin layer of each is covered by a sub-microscopic surface secretion of similar properties.

What has been said of the cuticles of the venter, the pleura, and the intersegmental membranes of *Epicoccus* sp., applies equally to those of the same regions of *E. acaciae*, such differences as do occur being confined to the dorsal procuticle.

The endocuticle of the latter has undergone still further reduction and forms irregular cappings to terminations of the cuticular ingrowths. It does not occur elsewhere. The procuticle thus consists almost entirely of mesocuticle which structurally resembles that of *Epicoccus* sp.; its distinguishing characters are chemical. Millon's reagent colors it uniformly cherry-red—a much deeper shade than is produced by the reagent in insect cuticle generally. The xanthoproteic test stains it uniformly deep orange, the iodine technique purple-black, ninhydrin violet-pink, and Mallory's PTAH deep purple. Seyki's technique displays the pore canals as deep violet, filamentous tubes of approximately constant diameter throughout their length and demonstrates their continuity between the hypodermis and the outer procuticular surface. After twelve hours' extraction at 60° C. with 10% hydrochloric acid, Millon's reagent stains the whole procuticle pink, and the xanthoproteic test colors it yellow. It fails to give visible responses with the other stains and reagents.

These results would seem to indicate that extra protein is present in the mesocuticle, that it is uniformly distributed (in contrast to its aggregated condition in *Epicoccus* sp.), and that it is differentiated into acid-extractable and acid-resistant fractions.

A lipoid zone, whose extent and reactions are identical with those of the corresponding zone of *Epicoccus* sp., is present. It differs in being brown pigmented with melanin or a melanin-like product, and is hence easily recognizable even in unstained sections.

IV. The Wax Glands and Their Secretion

(a) *P. adonidum*

Wax glands are conspicuous in most sections of *P. adonidum*, frequently as many as four or five, transected in various places, being visible in the one section. They resemble those of *P. martinus* as described by Pollister (1937) and it is probable that a general uniformity of glandular structure prevails throughout the family. Fundamentally, each consists of a multicellular secretory sheath enclosing a large,

sub-spherical, central reservoir which communicates with the surface by means of one or more ducts (Fig. 1A).

When sections of unfixed material are treated with Sudan black B, the surface wax, the contents of reservoirs and ducts, and limited parts of the cuticle surrounding the ducts, are blackened. The local cuticular impregnation appears to be brought about by diffusion of some of the wax through the duct walls. The Nile blue sulfate technique stains the surface wax, that filling the ducts, and the impregnated cuticle deep blue, but it colors the reservoir contents red. Fat solvents readily dissolve the surface wax and that of the ducts but have no apparent effect on the impregnated cuticle or the reservoir material. The latter is highly resistant to such solvents; prolonged extraction with methanol-chloroform, pyridine, ether, or boiling cyclohexane removes part only of its lipoid. This explains why even in paraffin sections cut at 1 μ the contents of the reservoirs are retained apparently unaltered by the treatment they have undergone in the course of their preparation.

These results suggest that the material in the reservoir is "protowax" consisting of neutral lipoid (indicated by the Nile blue sulfate technique) so bound to other substances as to render its extraction extremely difficult. Alternatively the protowax may be secreted as an emulsoid whose finely divided lipoid micelles are dispersed in an inert medium. By some means at present unknown, the lipoid is freed and passed along the ducts. On its way to the surface the neutral lipoid becomes acidic, in which form it is deposited as wax on the surface. This interpretation of the course of events is purely speculative. An important obstacle to its acceptance is the failure of any of the wide range of tests applied, to demonstrate the presence in the reservoir of anything except lipoid.

There is some evidence suggesting that the outer surface of the wax layer may be covered by a sub-microscopic layer of protective material.

(a) If insects are killed with cyanide and then immersed in a solution of Sudan black B, the surface wax is stained only where it has been damaged during manipulation. If they are first lightly brushed, the areas so treated stain rapidly.

(b) If the insects have a prior immersion for fifteen minutes in 10% hydrochloric acid at 35° C. before being put in Sudan black B, staining of the wax is rapid and complete. Moreover, from insects so pre-treated, cold cyclohexane dissolves the wax rapidly whereas it acts much more slowly on untreated insects.

(c) If the insects are dropped into water at 70° C., the wax oozes away forming a surface film on the water. If such insects are then embedded in paraffin and sectioned, the epicuticle, when viewed under phase-contrast conditions, has an outer surface which cannot be sharply focussed. If the same sections are then treated with warm 10% hydrochloric acid for ten minutes, and re-examined, the epicuticle has a well-defined outer boundary.

Should an outer layer be present, the wax and its protective layer would afford an interesting analogy to the two outer layers of the four-layered type of epicuticle. They would occupy the same anatomical position relative to the two inner layers, and apparently perform the same function of limiting water loss as do the wax and cement layers. They differ in that the wax at least is a glandular product, they are not secreted until after moulting, and finally, some of the wax is used by many species as a covering for egg masses.

(b) *Epicoccus*

In *Epicoccus* most of the wax glands have atrophied. Their orifices are still open, but either their ducts are internally sealed off by the growth of procuticle across them, or their secretory cells are small and produce little wax. Glands of the latter kind are confined to the dorsum where they open in small groups in deep infolds of the cuticle adjoining muscle insertions. They secrete little more wax than is needed to plug the ducts and keep them filled. Their orifices are marked by small white spots at the surface (Fig. 4).

Typical glands are confined to the lateral extremities of the lobes in contact with the bark. The gland contents and the cementing wax respond similarly to stains and tests as do those of *P. adonidum*. The principal differences between the waxes of the two species are that that of *Epicoccus* has a higher melting point, and dissolves easily and quickly in all fat solvents. It would appear that the wax having ceased to function as an agent for reducing desiccation, no protective layer covers it.

V. Discussion

The results of this investigation demonstrate that notwithstanding the fundamental similarity of cuticular structure which prevails throughout the Pseudococcidae, great morphological differences distinguish the two genera studied.

A. The Epicuticle

The epicuticles of all three species consist of the essential cuticulin and paraffin layers. The paraffin layer of *Epicoccus* is overlain by a sub-microscopic covering of apparently lipoidal material which probably corresponds to the wax layer of more complex epicuticles, as a cement layer in this anatomical position would be abnormal. There is no indication of the presence of any such layer in *Pseudococcus* in which it is replaced by a thick layer of wax secreted by hypodermal glands; this surface wax itself appears to possess an extremely tenuous protective covering.

The effectiveness of either of these systems as a means for restricting water loss is probably slight. Much of the surface wax of *Pseudococcus*, for example, is disposed in long filaments which would have little value in this regard, more especially for a species which inhabits a humid micro-environment. Dead insects of either genus, after removal of the surface layer, do not lose water to a dry atmosphere at a significantly greater rate than do dead intact ones.

B. The Procuticle

The procuticles of the two genera differ as greatly as do the environments inhabited by each. The thin procuticle of *Pseudococcus*, consisting almost solely of endocuticle, displays little specialization. That of *Epicoccus*, on the contrary, is highly specialized both structurally and chemically.

In absolute thickness it is comparable with those of large sclerotized forms such as *Periplaneta*, while relative to the individual's size, there are probably few other insects which can match it. It consists almost wholly of mesocuticle.

Among insects whose cuticles have been described, that of *Epicoccus* is unique in the high proportion of protein in the procuticle, aggregated in a well-defined zone.

Its possession of a deep-seated lipoid zone in the procuticle is, so far as is known, shared only by the desert locust, *Schistocerca gregaria*.

All this specialization betokens a long period of evolution under conditions adverse to insect life generally, and the acquisition of a massive cuticle, together with the loss of mobility by the adult female, are presumably closely linked with this. Incapacity for locomotion may be disadvantageous; but failure to cope with a hostile environment spells extinction.

The collected material came from a single half-dead plant, not over ten feet in height. Fully-exposed as the insects were, they not infrequently had to contend with direct sun temperatures of 60° C., or even more, accompanied by relative humidities often below 10%, in a situation commonly swept by parching winds. The brood chambers of mature females contained eggs, and first and second nymphal instars. These immature forms were found nowhere else. Apart, therefore, from ensuring survival of the mother, the thick leathery cuticle functions equally well in protecting those stages in the life cycle most vulnerable to the environmental conditions, thereby making it possible for the species to maintain itself in a region relatively-poor in the higher forms of insect life.

I wish to thank Miss Helen M. Brookes of this department for supplying the material used and for allowing publication of the photograph reproduced in Figure 4.

My thanks are also due to Mr. Keith P. Phillips (in charge of Photographic Department) who is responsible for all the photography.

Summary

1. The cuticular structure of three female pseudococcids, *Pseudococcus adonidum* L., *Epicoccus* sp., and *E. acaciae* (Maskell), has been investigated.

2. The cuticle of *P. adonidum* consists of a two-layered epicuticle, overlying a thin procuticle, almost all of which is endocuticle.

3. The cuticle of *Epicoccus* sp., is highly specialized. Its epicuticle closely resembles that of *P. adonidum*. The dorsal cuticle is relatively thick, and is much modified chemically. Most of it consists of mesocuticle in which Millon's reagent delimits three well-defined zones which differ greatly in their reactions to stains and histochemical reagents. The endocuticle is much reduced. A thin layer of procuticle between the hypodermis and the endocuticle is impregnated with lipoid to from a "lipoid zone."

4. The cuticle of *E. acaciae* is thick. It differs from that of *Epicoccus* sp. principally in that there is no chemical zonation of the procuticle, and the lipoid zone is melanin-pigmented.

5. Wax glands are numerous in the cuticle of *P. adonidum*. The contents of their reservoirs ("protowax") differ chemically from the surface wax. In *Epicoccus*, many of the glands have atrophied; typical glands are confined to lateral parts of the cuticle in contact with the host plant, and these secrete large quantities of wax which fixes the insect permanently in position.

6. The specialized cuticle of *Epicoccus* appears to have evolved over a long period, during which the insects have been exposed to adverse environmental conditions.

LITERATURE CITED

DENNELL, R., 1946. A study of an insect cuticle: the larval cuticle of *Sarcophaga falculata* Pand. (Diptera). *Proc. Roy. Soc. London, Ser. B,* **133**: 348–373.

DENNELL, R., AND S. R. A. MALEK, 1955. The cuticle of the cockroach *Periplaneta americana* II The epicuticle. *Proc. Roy. Soc. London, Ser. B,* **143**: 239–257.

LOWER, H. F., 1956. The terminology of the insect cuticle. *Nature,* **178**: 1355–1356.

LOWER, H. F., 1957a. The acellular coverings of the immature stages of *Aphodius howitti* Hope (Coleoptera: Scarabaeidae). *J. Morph.* (in press).

LOWER, H. F., 1957b. Iodophil components of insect cuticle. *Stain Tech.,* **32**: 127–129.

MALEK, S. R. A., 1956. An ecdysial membrane in the locust cuticle. *Nature,* **178**: 1185–1186.

POLLISTER, P. F., 1937. The structure and development of wax glands of *Pseudococcus maritimus* (Homoptera, Coccidae). *Quart. J. Micr. Sci.,* **80**: 127–148.

SCHMIDT, E. L., 1956. Observations on the subcuticular layer in the insect integument. *J. Morph.,* **99**: 211–226.

WIGGLESWORTH, V. B., 1947. The epicuticle in an insect, *Rhodnius prolixus* (Hemiptera). *Proc. Roy. Soc. London, Ser. B,* **134**: 163–181.